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Development of muffin by incorporation of dried *Moringa oleifera* (Drumstick) leaf powder with enhanced micronutrient content

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Abstract
Nutritional enhancement to improve the functionality of food products has gained momentum over the years. Therefore, identification and incorporation of inexpensive, easily available food sources with rich nutrients, antioxidants and medicinal benefits into different food products would serve as a big boon to the food industry. The dried leaves of *Moringa oleifera* (Drumstick tree) are one such source which holds great promise. For exploring its potential, the present study involved the incorporation of dried *Moringa oleifera* leaf powder (MOLP) into a popularly consumed food product like Muffin. Through the study, product optimization, analysis of physicochemical and functional content and storage study (sensory and microbiology) of *moringa* muffin was done in comparison with the control muffin. The product optimization with different concentrations of dried MOLP was done on the basis of preliminary (5%, 10% and 15%) and final (8%, 10% and 12%) sensory analysis (color & appearance, odor, texture, flavor and overall acceptability) with 9-point hedonic scale. The results concluded that muffin with 12% concentration was most preferred. The physicochemical and functional tests of optimized *moringa* muffin depicted a significant increase ($p<0.05$) in moisture (17.67%), ash (1.63%), protein (7.5g/100g), fat (15.04g/100g), iron (3.55mg/100g), calcium (55.06mg/100g), potassium (111.03mg/100g), beta carotene (12.999mg/100g) and vitamin C (37.5mg/100g) than the control muffin.

Keywords: Dried *Moringa oleifera* powder, Moringa muffin, product optimization, physicochemical, sensory and microbiological analysis

1. Introduction
'Moringa oleifera' is commonly known as Drumstick tree and is a part of the family called Moringaceae. There are 33 species of the Moringaceae family [1]. The drumfstick tree is a small fast-growing tree which is native to India. The origin of the tree is said to be from Agra and Oudh in North Western region of India to South of the Himalayan Mountains. They are cultivated in Asian, African, Middle Eastern and South American regions [2]. The most suited soil conditions are dry sandy soil where these trees grow to their full potential. But they are also propagated in semi-arid, tropical and subtropical regions [3]. Being drought resistant, they are able to withstand a wide range of soil and rainfall conditions and are therefore available throughout the year [2]. Drumstick tree can prove to play an important role in climate change mitigation as it has the ability to absorb carbon dioxide, thereby decreasing the amount of CO_2 in the atmosphere and preventing global warming and ozone layer depletion [4]. *M. oleifera* is an excellent food source for human beings and is a multipurpose tree which can also be promoted as a fodder crop [5]. Several other uses include medicine, dye, nutritional and industrial applications [6]. *Moringa oleifera* seeds can be used for water treatment as it can remove up to 99% bacteria from water and purify it [7]. Studies report the multipurpose use of different parts of drumstick tree in food for human consumption such as yogurt, cake, bread, biscuits and soup [6]. Fresh *Moringa* leaf juice can be used as a growth hormone to increase the crop yields by 25-35% [7].
M. oleifera leaves are also commonly used as a replacement for spinach in curries and vegetable dishes. The leaves can be dried, ground into a powder and stored without refrigeration conditions for many months and without loss of nutrients. The dried powder is completely edible and is used in soups and a variety of traditional foods. The leaves are quite versatile as they can be eaten fresh, cooked, or stored as dried powder. The powdered leaves are packed with nutritional properties as they are a rich source of protein (23.78g/100g), fiber (11.8g/100g), Beta carotene (37800µg/100g), vitamin C (56g/100g) and minerals like calcium (3467mg/100g), iron (19mg/100g), phosphorous (215mg/100g) [3] and potassium (1467mg/100g) [8]. *M. oleifera* contains 7, 17, 10, 9, 25, 15 times the amount of vitamin C in oranges, calcium in milk, vitamin A in carrots, protein in yogurt, iron in spinach and potassium in bananas respectively [9].
The protein content of the dried MOLP is similar to that of moth beans, soybeans and kidney beans which have (22 - 24%) protein. Pulses being expensive are difficult to purchase by the poor people of the developing countries therefore dried MOLP can be used in food products to serve the role of pulses [3]. There are ongoing research studies on *Moringa oleifera* in South Africa on its use to eradicate malnutrition, especially among the disadvantaged communities [10]. Organizations like WHO (World Health organization) also encourage the use of *Moringa oleifera* as a source to tackle malnutrition [11].
Along with nutritional properties, the leaves are loaded with medicinal benefits as they possess flavonol and phenolic acid which have the ability to inhibit prostate cancer and breast cancer [3]. Root, bark, flower and leaf are used for treatment of infectious diseases along with cardiovascular and gastrointestinal disorders [12]. The leaves also perform various pharmacological activities in our system, such as analgesic, antihypertensive, antitumor activity, and anti-inflammatory

effects [2]. The calorie content in *M. oleifera* leaves is also less, making it suitable for obese patients [13]. It has been found that the leaves contain natural antioxidants such as vitamin C, tocopherols, flavonoids and other phenolic compounds. The total flavonoid content (TFC) and the total phenolic content (TPC) of *M. oleifera* leaves are quite higher than some of the common vegetables like spinach and broccoli [10].

Potential use of *Moringa oleifera* leaves

M. oleifera leaves have higher radical scavenging ability than some vegetables, therefore, serving as a better choice of antioxidant than common vegetables. It is recommended to use these leaves in food products to enhance the nutritive value. One of the best ways of utilization of these leaves is to dry them first and then incorporate them into various food products. Leaves particularly when dried are easy to handle and store as they have a very good shelf life. Also, after drying, the nutrients are more concentrated, thereby making them even richer and more valuable [10]. Studies have been conducted on fortification of *Moringa* leaf powder in chocolate and seasame sweet, supplementation of wheat flour with *M. oleifera* powder in bread and effect of drumstick leaves extract on nutritional quality of *moringa* paneer [14, 15, 16].

The current study involved the incorporation of dried MOLP in muffin batter to make *moringa* muffin. Bakery products form important staple foods in most countries and cultures, providing us with most of our food calories. They are also an essential part of a balanced diet [17]. One of the most relished bakery items by all age groups is muffin. The trend for development of functional muffins has grown over the years. In order to target a large consumer base, the current study was done to assess the feasibility of producing an acceptable muffin with improved nutrition by incorporation of dried MOLP.

2. Materials and Methods

All the ingredients (refined wheat flour, eggs, castor sugar, butter, milk, baking powder, vanilla essence) for preparation of *moringa* muffin were purchased from local market. Fresh *Moringa oleifera* leaves were procured from the trees in Dwarka, New Delhi, India.

Preparation of dried MOLP

Drying of fresh *Moringa oleifera* leaves was carried out, with slight modifications, following the method reported by Joshi and Mehta [3]. Fresh, undamaged leaves were sorted and washed with clean water thoroughly to remove all dirt. The water was drained and leaves were air dried for some time. The washed and air-dried leaves were then dried in a tray drier at 60°C for 4 hours. The dried leaves were ground, sieved (80 mesh size), packaged in zip lock bags and stored (-18°C).

Product development

Moringa muffins were made by incorporating 12% dried MOLP in refined wheat flour. The process was followed with slight modification from the recipe given in "The art and science of cooking" [18].

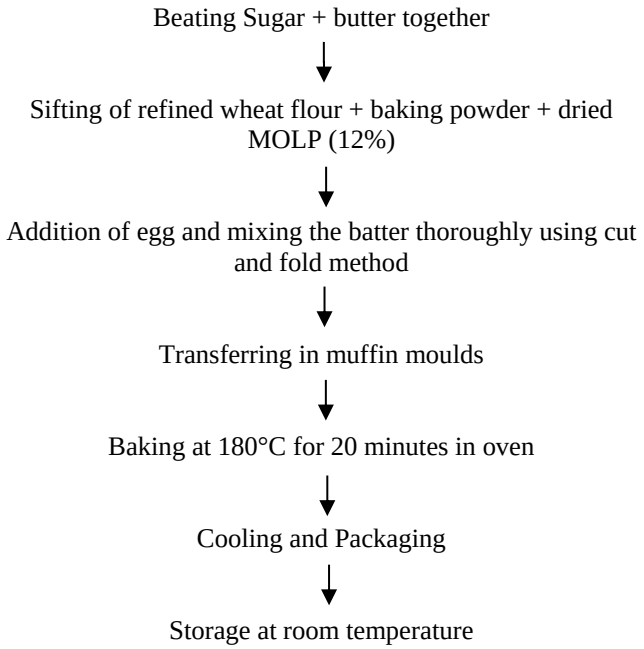

Fig 1: Flowchart for making Moringa Muffin

Physicochemical and functional analysis

Moisture, ash, protein, fat were analyzed as per the standard methods given in AOAC [19]. Iron, phosphorous and vitamin C were determined by the method given in Rangana [20]. Calcium was estimated using Atomic Absorption Spectrophotometer (AAS) (IS: 13433 (P1) 1992) as described by Indian standards [12]. Potassium was determined by flame photometry [22] and Beta carotene was determined by UPLC (Ultra Performance Liquid Chromatography).

Sensory evaluation (9 point hedonic score card)

Sensory evaluation of the control and formulated product was done using a semi-trained panel of 30 members. The panelists scored on the basis of Color and appearance, odor, flavor, texture and overall acceptability on a 9-point hedonic scale as described by FAO (Food and Agriculture Organization) [23].

Microbiological analysis

The microbiological tests were conducted on the samples by referring the procedure given for Microbial analysis of Food products in Microbiology laboratory manual, Cappuccino [24] with modifications.

Statistical analysis

The data generated with triplicate readings were studied using one-way analysis of variance (ANOVA). Mean values were tested for significant difference using Duncan Post Hoc test (SPSS Software). Values with $p < 0.05$ were considered statistically significant.

3. Results and Discussion

Incorporation and optimization of the amount of dried MOLP in muffin through sensory analysis

The concentration of dried MOLP to be incorporated in muffin was optimized in two stages namely, preliminary and final stage. For preliminary screening, a wider range of concentration (5%, 10% and 15%) of dried MOLP was taken.

Sensory evaluation (9-point hedonic scale) on parameters like color and appearance, taste, smell (odor), mouthfeel and overall acceptability. Table 1 depicts mean scores for all sensory parameters. It was observed that muffin with 10% concentration of dried MOLP had significantly higher scores ($p<0.05$) for taste, mouthfeel and overall acceptability than its counterparts, 5% and 15%. Suggesting, 10% concentration of dried MOLP in muffin was most preferred by sensory panel members. Therefore, for final optimization of concentration of dried MOLP in muffin, a concentration lower (8%) and a concentration higher (12%) was taken along with 10% concentration.

The results for final sensory analysis are depicted in Table 1. It can be seen that out of the three concentrations, 12% concentration of MOLP was most preferred by the sensory panelists as per the mean scores. A significantly ($p<0.05$) higher scores were observed for muffin with 12% dried MOLP for odor, taste and overall acceptability. The overall acceptability score was 7.38 for 8%, 7.25 for 10% and 8.05 for 12%. Therefore, 12% concentration of dried MOLP in muffin was selected as the optimum concentration to be incorporated in the final product. Similar study conducted by Dachana *et al.* [25] indicated that the cookies incorporated with 10% of dried *Moringa* leaf powder were acceptable by sensory panelists.

Table 1: Sensory analysis scores for optimization of levels of dried MOLP in muffin (preliminary and final screening levels)

	Concentration of dried MOLP	Color and appearance	Smell (Odor)	Taste	Mouth feel	Overall acceptability
Preliminary Screening	5%	$7.75^{a}\pm0.164$	$7.13^{b}\pm0.295$	$7.00^{b}\pm0.189$	$7.00^{b}\pm0.189$	$7.25^{b}\pm0.164$
	10%	$8.00^{a}\pm0.000$	$8.00^{a}\pm0.267$	$7.75^{a}\pm0.164$	$8.13^{a}\pm0.295$	$8.00^{a}\pm0.189$
	15%	$7.25^{b}\pm0.164$	$6.75^{b}\pm0.250$	$6.63^{b}\pm0.263$	$7.25^{b}\pm0.164$	$7.13^{b}\pm0.125$
Final Screening	8%	$6.88^{b}\pm0.295$	$6.63^{b}\pm0.263$	$6.75^{b}\pm0.250$	$7.00^{a}\pm0.189$	$7.38^{b}\pm0.183$
	10%	$7.95^{a}\pm0.164$	$7.13^{b}\pm0.295$	$7.00^{b}\pm0.189$	$7.50^{a}\pm0.189$	$7.25^{b}\pm0.164$
	12%	$8.12^{a}\pm0.125$	$7.86^{a}\pm0.267$	$7.88^{a}\pm0.263$	$7.78^{a}\pm0.263$	$8.05^{a}\pm0.267$

#[ab] Means in a row for the samples with the same letter are not significantly different ($p>0.05$)
#the values represent mean ± standard error from 10 determinations

Physicochemical and Nutritional analysis of control and optimized moringa muffin

The physicochemical and nutritional analysis of control muffin and *moringa* muffin is depicted in Table 2. The moisture content increased significantly ($p<0.05$) from 14% in control muffin to 17.67% in *moringa* muffin. This may be due to increase in protein content in *moringa* muffin. Bakery products show an increase in moisture content with increase in protein content [26]. The ash content of *moringa* muffin showed a significant increase ($p<0.05$). The higher ash content of *moringa* muffin indicates higher amount of minerals in it which may be attributed by dried MOLP. Mouminah [27] also reported a significant enhancement ($p<0.05$) in ash content of *moringa* cookies. The protein and fat content increased significantly ($p<0.05$) from 6.98g/100g in control muffin to 7.5g/100g in *moringa* muffin and 12.11g/100g in control muffin to 15.04g/100g in *moringa* muffin respectively. Increase in protein content may be because dried MOLP is a rich source of protein [28]. Beta carotene in *moringa* muffin was observed to be 12.999 mg/100g. Sengev *et al.* [15] reported a significant increase in beta carotene and protein with increase in levels of *Moringa oleifera* leaf powder supplemented in bread. Comparable value of beta carotene (16.3 mg/100g) was reported by Fuglie [29] for dried *Moringa oleifera* leaf powder. A significantly high value ($p<0.05$) was obtained for vitamin C content indicating dried MOLP is a rich source of vitamin C [12], thereby helping in iron absorption in animal's body [30]. Mineral content improved tremendously upon incorporation of dried MOLP in muffin. Calcium, potassium and iron showed a significant enhancement ($p<0.05$) in *moringa* muffin, however, phosphorous content increased but not significantly ($p>0.05$). Sengev *et al.* [15] also reported a significant increase in mineral content upon increasing the levels of supplementation of wheat flour with *Moringa oleifera* leaf powder. The increased amount of minerals in *moringa* muffin is attributed by *Moringa* leaves which upon drying contain concentrated and rich source of minerals [27, 3].

Table 2: Physicochemical and Nutritional analysis of Control and *Moringa* Muffin

Parameter	Control muffin	*Moringa* muffin
Moisture (%)	$14^{a}\pm2.08$	$17.67^{b}\pm1.76$
Ash (%)	$0.98^{a}\pm0.005$	$1.63^{b}\pm0.12$
Protein (g/100g)	$6.98^{a}\pm0.04$	$7.5^{b}\pm0.15$
Fat (g/100g)	$12.11^{a}\pm0.01$	$15.04^{b}\pm0.03$
Beta carotene (mg/100g)	-	12.99
Vitamin C (mg/100g)	$14.28^{a}\pm2.74$	$37.50^{b}\pm4.33$
Calcium (mg/100g)	$26.22^{a}\pm0.11$	$55.06^{b}\pm0.01$
Potassium (mg/100g)	$59.18^{a}\pm0.01$	$111.03^{b}\pm0.01$
Phosphorous (mg/100g)	$225.0^{a}\pm5.00$	$275.0^{a}\pm2.50$
Iron (mg/100g)	$2.65^{a}\pm0.12$	$3.55^{b}\pm0.25$

#[ab] Means in a row for the samples with the different letter are significantly different ($p<0.05$)
#the values represent mean ± standard error from three determinations

Changes in sensory scores and microbial count over storage for 7 days at room temperature (RT) (28ºC)

Sensory changes

The changes in sensory scores for control and *moringa* muffin for 7 days (0, 1, 2, 3, 4, 5, 6 days) are represented in Table 3 and Figure 3. The sensory scores for color and appearance, odor, flavor, texture and overall acceptability decreased over storage for 7 days. No significant difference ($p>0.05$) was observed between control muffin and *moringa* muffin for each day. However, scores significantly decreased ($p<0.05$) with each day in control muffin as well as *moringa* muffin. The score of color and appearance was slightly higher for control muffin than moringa muffin. The mean scores for flavor, texture, odor and overall acceptability was higher in case of *moringa* muffin in totality for 7 days. The addition of sugar and vanilla essence would have prevented *moringa* muffin

from having the characteristic herbal flavor and odor of *Moringa* leaves. The texture of *moringa* muffin was better as it was soft yet crumbly compared to control muffin throughout 7 days, however both samples showed signs of degradation as they became soggy from day 8 onwards. The overall acceptability scores for 7 days being consistently higher for *moringa* muffin indicates that panelists accepted muffin incorporated with dried MOLP. The results of the study conducted by Abbas and Jayasena [31] showed that the storage period of muffin incorporated with lupin flour for 7 days caused substantial changes in the texture of muffin.

Table 3: Changes in sensory scores (color and appearance, odor, taste, mouthfeel and overall acceptability) of control and *moringa* muffin over 7 days of storage at room temperature (28ºC)

Color and appearance			**Flavor**		
Days	**Control**	***Moringa* Muffin**	**Days**	**Control**	***Moringa* Muffin**
0	$8.267^{aA} \pm 0.18$	$8.333^{aA} \pm 0.17$	0	$7.967^{aA} \pm 0.18$	$8.367^{aA} \pm 0.14$
1	$8.000^{aAB} \pm 0.15$	$8.100^{aAB} \pm 0.17$	1	$7.900^{aAB} \pm 0.15$	$8.167^{aAB} \pm 0.20$
2	$7.967^{aAB} \pm 0.17$	$7.967^{aAB} \pm 0.18$	2	$7.800^{aAB} \pm 0.21$	$8.167^{bAB} \pm 0.17$
3	$7.967^{aAB} \pm 0.17$	$7.633^{bBC} \pm 0.22$	3	$7.733^{aAB} \pm 0.18$	$7.883^{aBC} \pm 0.17$
4	$7.783^{aABC} \pm 0.14$	$7.567^{aBC} \pm 0.21$	4	$7.633^{aAB} \pm 0.19$	$7.617^{aBC} \pm 0.20$
5	$7.667^{aBC} \pm 0.24$	$7.600^{aBC} \pm 0.15$	5	$7.333^{aBC} \pm 0.22$	$7.417^{aCD} \pm 0.18$
6	$7.300^{aC} \pm 0.19$	$7.333^{aC} \pm 0.17$	6	$7.000^{aC} \pm 0.24$	$7.067^{aD} \pm 0.22$
Texture			**Odor**		
Days	**Control**	***Moringa* Muffin**	**Days**	**Control**	***Moringa* Muffin**
0	$7.930^{aA} \pm 0.18$	$7.830^{aAB} \pm 0.20$	0	$7.933^{aA} \pm 0.19$	$8.133^{bA} \pm 0.16$
1	$7.970^{aA} \pm 0.17$	$8.070^{aA} \pm 0.17$	1	$7.900^{aA} \pm 0.22$	$7.967^{aAB} \pm 0.23$
2	$7.700^{aA} \pm 0.21$	$7.970^{aA} \pm 0.23$	2	$7.867^{aA} \pm 0.23$	$7.867^{aABC} \pm 0.23$
3	$7.800^{aA} \pm 0.17$	$7.800^{aAB} \pm 0.17$	3	$7.600^{aAB} \pm 0.19$	$7.917^{bAB} \pm 0.15$
4	$7.400^{aAB} \pm 0.21$	$7.500^{aABC} \pm 0.21$	4	$7.400^{aAB} \pm 0.20$	$7.417^{aCD} \pm 0.21$
5	$7.130^{aBC} \pm 0.21$	$7.330^{aBC} \pm 0.20$	5	$7.433^{aAB} \pm 0.18$	$7.300^{aCD} \pm 0.22$
6	$6.800^{aC} \pm 0.17$	$7.070^{aC} \pm 0.21$	6	$7.000^{aB} \pm 0.21$	$7.133^{aD} \pm 0.20$
Overall acceptability					
Days	**Control**	***Moringa* Muffin**			
0	$7.983^{aA} \pm 0.18$	$8.300^{bA} \pm 0.17$			
1	$7.933^{aA} \pm 0.14$	$8.167^{aAB} \pm 0.16$			
2	$7.933^{aA} \pm 0.16$	$8.067^{aAB} \pm 0.17$			
3	$7.867^{aA} \pm 0.20$	$7.950^{aAB} \pm 0.17$			
4	$7.700^{aA} \pm 0.15$	$7.683^{aBC} \pm 0.19$			
5	$7.567^{aAB} \pm 0.19$	$7.683^{aBC} \pm 0.15$			
6	$7.150^{B} \pm 0.19$	$7.383^{aC} \pm 0.17$			

^{ab}Means in a row for an attribute with the same letter are not significantly different ($p>0.05$); ^{AB}Means in a column for an attribute with the same letter are not significantly different ($p>0.05$)

#the values represent mean ± standard error from 30 observations

Microbiological changes in control and moringa muffin over storage

Muffin samples were plated on nutrient agar plates on 0th, 2nd, 4th and 7th day for observing the change in bacterial count. For fungi, muffin samples were plated on potato dextrose agar plates on 0th day observed after a week. Different dilutions (10^{-2}, 10^{-4}, and 10^{-6}) were plated for 0th day, 2nd, 4th and 7th day. Number of colonies was too low to count for 0th, 2nd and 4th day. This low count might be due to high temperature used for baking the muffins. On 6th day, dilutions of 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8} were plated and it was observed that colony count were too numerous to count in lower dilution but in higher dilution (10^{-8}) countable number of colonies were observed. Since muffins started becoming soggy due to moisture absorption from environment the colony count must have increased. Moisture provides desirable conditions for microbes to thrive [2]. The Cfu/ml (Colony forming unit) value for control and *moringa* muffin was $8.9*10^{10}$ and $7.45*10^{10}$ respectively for day 6. According to Centre of Food Safety, Food and Environmental Hygiene Department [32], a cfu/ml value $\geq 10^6$ for bakery and confectionary products is considered unsatisfactory. Yeast growth was not prominent after 7 days in both control and *moringa* muffin. Due to too numerous growth of bacteria in samples which may be due to lack of aseptic packaging; sensory analysis was discontinued. Microbiological study conducted by Neelam, Ramesh and Madhulika [33] indicate that muffin substituted with apple skin powder when kept in LDPE pouches were safe for consumption for 21 days. Therefore, proper packaging would enable moringa muffin to remain safe for consumption for more days.

4. Conclusion

From the present study, it may be concluded that incorporation of dried MOLP in muffin up to a concentration of 12% in refined wheat flour would be successful in producing a muffin with improved nutrition along with acceptable sensory

attributes. Consumption of such foods will be a way to tackle nutritional deficiencies existing among the population and also help in boosting the overall health of an individual. *Moringa oleifera* being inexpensive and easily available is a domain offering the possibility for more research to utilize its leaves and other parts to full potential as an ingredient in different food products so that there is increased awareness and application of this wondrous tree.

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